



TITLE:

Diesel exhaust particles suppress pathogen-associated molecular pattern-induced cytokine generation from bronchial epithelial cells

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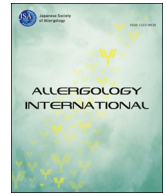
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Letter to the Editor

Diesel exhaust particles suppress pathogen-associated molecular pattern-induced cytokine generation from bronchial epithelial cells



Dear Editor

Air pollutants have been shown to be involved in the pathogenesis of various respiratory diseases, including asthma. Diesel exhaust particles (DEP) are the main constituents of particulate matter (PM) 2.5, which consists of PM with a diameter of less than 2.5 μm . Epidemiological studies have revealed that DEP is responsible for a recent increase in airway allergic disease. Diesel exhaust particles exert an adjuvant effect on IgE production, enhance airway hyperresponsiveness,¹ and potentiate airway remodeling in experimental asthma models.

Epidemiological studies have suggested that in addition to their relationship with asthma, air pollutants cause susceptibility to respiratory infections. Animal studies have shown that DEP pretreatment enhances susceptibility to various pathogens, but the precise mechanisms remain unclear. Although bronchial epithelial cells play important host-defensive roles against pathogens by secreting various cytokines, the effect of DEP on pathogen-associated molecular pattern (PAMP)-induced cytokine generation has not been comprehensively investigated. To elucidate the involvement of epithelial cells in DEP-induced susceptibility to pathogens, we investigated the effect of DEP on PAMP-induced cytokine generation from bronchial epithelial cells.

A human bronchial epithelial cell line, BEAS-2B cells, were cultured as described previously² and stimulated by previously described Toll-like receptor (TLR) ligands.³ BEAS-2B cells were plated on 96-well culture plates (Iwaki Glass) in DMEM/Ham's F12K medium containing 5% of FCS. Before stimulation, the culture medium in each well was replaced by an identical formulation containing 0.1% BSA instead of FCS, as previously described.² BEAS-2B cells were stimulated for 48 h by TLR2 ligand: Pam3CSK4 (Pam3, EMC Microcollections, Tübingen, Germany), TLR3 ligand: poly I:C (virus double-stranded RNA analogue, Sigma–Aldrich, St. Louis, MO, USA), or TLR4 ligand LPS (O26:B6, Sigma–Aldrich), and the levels of cytokines in the culture supernatant were simultaneously determined with a bead array system using Bio-Plex Pro human cytokine 27-Plex (#M50-OKCAF0Y, BIO-RAD, Hercules, CA, USA) and Luminex (Luminex Japan Co., Tokyo, Japan). The effect of pretreatment with 1–100 $\mu\text{g}/\text{ml}$ of DEP for 2 h was also analyzed. Diesel exhaust particles were prepared as previously described.⁴ The concentration of 100 $\mu\text{g}/\text{ml}$ DEP used in this study is achievable in a period of 1 day in Los Angeles and does not show cytotoxicity to BEAS-2B, as shown by trypan blue dye exclusion.⁵ All

data are expressed as mean \pm SEM ($n = 4$). Differences between values were analyzed by one-way ANOVA test, followed by Fisher's protected least significant difference test to compare individual groups.

First, simple stimulation by Pam3 and poly I:C without DEP pretreatment dose-dependently induced the generation of inflammatory (TNF- α , IL-1 β), regulatory (IL-10, Fig. 1, white columns), Th1 (IFN- γ), and Th2 (IL-4, IL-13) cytokines (Fig. 2, white columns). These PAMPs also induced dose-dependent generation of G-CSF, GM-CSF, IL-2, and IL-12 (data not shown). LPS induced generation of IL-1 β , IL-10 (Fig. 1), IFN- γ , and IL-4 (Fig. 2) at the highest dose. DEP alone induced only IL-10 generation (Fig. 1, Nil).

Next, we pretreated BEAS-2B with DEP for 2 h and subsequently stimulated with PAMPs. Pretreatment with DEP at 1 or 10 $\mu\text{g}/\text{ml}$ upregulated the Pam3-induced secretion of TNF- α , IL-1 β , IL-10 (Fig. 1, black columns), IFN- γ , and IL-4 (Fig. 2, black columns). In contrast, a higher dose of DEP significantly suppressed the generation of those cytokines at 100 $\mu\text{g}/\text{ml}$. In case of poly I:C stimulation, 1 or 10 $\mu\text{g}/\text{ml}$ of DEP did not upregulate cytokine secretion, but high-dose DEP significantly suppressed generation of cytokines other than IL-10 and IL-13 (Fig. 1, 2). High-dose DEP also suppressed LPS-induced IL-1 β , IFN- γ , and IL-4 generation (Fig. 1, 2).

We also analyzed the effect of DEP pretreatment on the ratio of IFN- γ /IL-13 concentration (Fig. 2, lowest column). After simple stimulation with Pam3, Poly I:C, and LPS, the ratio was elevated and formed IFN- γ dominant circumstances. In contrast, high-dose DEP strongly suppressed IFN- γ generation as compared with IL-13. The IFN- γ /IL-13 ratio decreased and an IL-13 dominant status emerged.

Depending on the experimental conditions, some studies have reported upregulated expression of IFN- γ or IFN- β after exposure to DEP in *in vitro* or *in vivo* studies. However, majority of the previous animal studies have shown that DEP pretreatment leads to elevation in the load of virus, *Mycobacterium*,⁶ and *Listeria*⁷ and decreases the local expressions of inflammatory cytokines, including TNF- α , IL-1 β , and IFN- γ . Other postulated mechanisms for DEP-induced susceptibility are attenuation of the level of host defensive Clara cell secretory protein, and oxidative stress-increased attachment of virus to epithelial cells. In case of *Listeria* infection, DEP decreased the production of inflammatory cytokines from alveolar macrophage.⁷ *Listeria* and *Mycobacterium* are recognized by TLR2, and in the present study, high-dose DEP suppressed cytokine generations from epithelial cells by the TLR2 ligand Pam3. Thus, in addition to macrophages, suppression of cytokine generations from bronchial epithelial cells may also contribute to the susceptibility to those pathogens.

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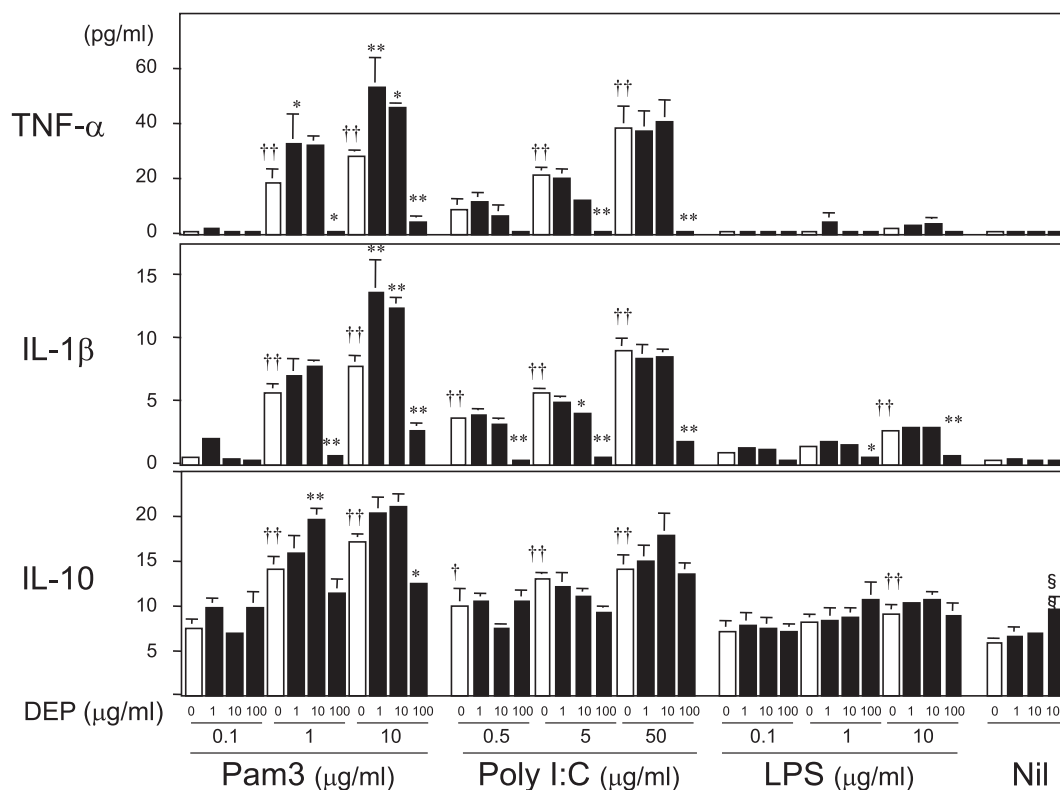


Fig. 1. Inflammatory and regulatory cytokine generation by BEAS-2B cells BEAS-2B cells were cultured with PAMP (Pam3, poly I:C, or LPS) for 48 h, with (black columns) or without (white columns) pre-treatment by 1–100 μ g/ml of DEP for 2 h. The levels of cytokines in culture supernatant are shown ($n = 4$). As control, BEAS-2B cells were cultured without PAMP (= Nil). $^{\dagger\dagger}p < 0.01$, $^{\dagger}p < 0.05$ vs. Nil (DEP 0 μ g/ml). $^{**}p < 0.01$, $^{*}p < 0.05$ vs. DEP 0 μ g/ml at the corresponding dose of PAMP. DEP alone induced only IL-10 generation ($^{ss}p < 0.01$ vs. DEP 0 μ g/ml, Nil). Assay sensitivity was as follows: TNF- α , 6.0 pg/ml; IL-1 β , 0.6 pg/ml; and IL-10, 0.3 pg/ml.¹³

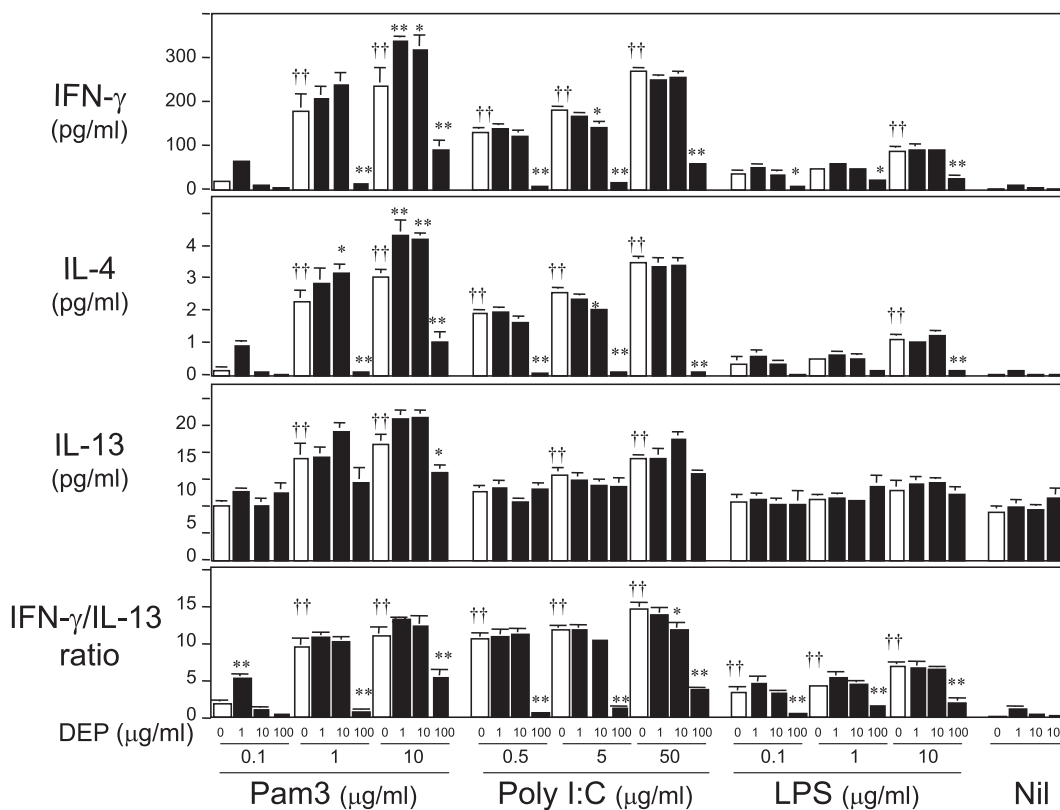


Fig. 2. Th1 and Th2 cytokine generation by BEAS-2B cells BEAS-2B cells were cultured with indicated PAMPs for 48 h, with (black columns) or without (white columns) pretreatment with DEP for 2 h. The levels of cytokines in culture supernatant are shown ($n = 4$). As control, BEAS-2B cells were cultured without PAMP (= Nil). $^{\dagger\dagger}p < 0.01$, $^{\dagger}p < 0.05$ vs. Nil (DEP 0 μ g/ml). $^{**}p < 0.01$, $^{*}p < 0.05$ vs. DEP 0 μ g/ml at the corresponding dose of PAMP. Assay sensitivity was as follows: IFN- γ , 6.4 pg/ml; IL-4, 0.7 pg/ml; and IL-13, 0.7 pg/ml.¹³

Although DEP has been reported to activate nuclear factor- κ B, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase, the precise effect of DEP on TLR signaling pathways is largely unknown. Diesel exhaust particles have been reported to enhance TLR3 expression and IFN- β secretion by poly I:C via IRF3 activation by DEP-induced Akt phosphorylation in A549 cells.⁸ Diesel exhaust particles also upregulate TLR4 expression.⁴ In the present study, low-dose DEP enhanced cytokine generation from epithelial cells by only Pam3, suggesting that the effect of DEP differs among TLRs. As TLR2, 3, and 4 have different adaptor proteins and downstream signaling cascades, further receptor-specific investigation of interaction is needed.

The most frequent cause of asthma exacerbation is infection, mainly by various viruses. Previous studies using an experimental asthma model has shown that DEP exaggerates allergic airway inflammation under stable asthmatic conditions.^{1,9} The underlying mechanisms include enhanced expression of local IL-13⁹ and decreased secretion of IFN- γ from NK and NKT cells,¹⁰ which lead to the formation of Th2-dominant circumstances. In addition to those findings, our present study suggested the role of DEP and epithelial cells in establishing IL-13-dominant status in PAMP-induced asthma exacerbation. DEP predominantly suppressed the generation of IFN- γ as compared with IL-13 and IL-10 from epithelial cells. The result suggested the detrimental role of chronic DEP exposure in worsening of allergic airway inflammation under various infections.

Although the concentration of some cytokines was low in this study, the expression of IL-13 and IFN- γ in bronchial epithelial cells was in the upper detection limit of the assay and in agreement with previous reports.^{11,12} Future studies will be conducted to confirm the expression of those cytokines at the mRNA level. In addition, the significance of bronchial epithelial-derived IL-13 and IFN- γ as compared to those derived from other inflammatory cells, could not be determined in the present study, and it merits further investigation in *in vivo* studies.

In conclusion, we found that DEP suppressed PAMPs-induced cytokine generation from bronchial epithelial cells. The result may in part explain the mechanisms of DEP-induced susceptibility to respiratory infections.

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Conflict of interest

The authors have no conflict of interest to declare.

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